

perature have a decidedly destructive effect upon the vitality and virulence of the organism.

I am very greatly indebted to Professor Dewar, F.R.S., not only for a constant supply of liquid air, but also for many valuable suggestions given me during the course of the experiment, and my cordial thanks are due to Dr. Roux and the officials of the Pasteur Institute for the facilities given me at that Institution for carrying out the necessary inoculations.

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“On the Behaviour of Oxy-hæmoglobin, Carbonic-oxide-hæmoglobin, Methæmoglobin, and certain of their Derivatives, in the Magnetic Field, with a Preliminary Note on the Electrolysis of the Hæmoglobin Compounds.” By ARTHUR GAMGEE, M.D., F.R.S., Emeritus Professor of Physiology in the Owens College, Victoria University. Received and Read, June 20, 1901.

1. *The Observations of Faraday and Plücker on the Diamagnetic Properties of the Blood.*

In the course of his investigations on magnetism and diamagnetism, read before the Royal Society in the year 1845, Faraday\* found that, notwithstanding the iron which its colouring matter contains, the blood is a diamagnetic liquid. “I was much impressed,” he remarked, “by the fact that blood was not magnetic, nor any of the specimens tried of red muscular fibre of beef or mutton. This was the more striking, because, as will be seen hereafter, iron is *always* and in almost all states magnetic. But in respect to this point it may be observed that the ordinary magnetic property of matter and this new property are in their efforts opposed to each other; and that when this property is strong it may overcome a very slight degree of ordinary magnetic force, just as also a certain amount of magnetic property may oppose and effectually hide the presence of this force.”† Faraday further found the blood to behave like all the constituent tissues of animal bodies which he investigated, and was led to state that “if a man could be suspended with sufficient delicacy, after the manner of Dufay, and placed in the magnetic field, he would point equatorially;

\* “On New Magnetic Actions and on the Magnetic Condition of all Matter,” ‘Phil. Trans.’, 1846, part 1.

† Faraday’s ‘Experimental Researches in Electricity,’ vol. 3 (1845), p. 36, para. 2285.

for all the substances of which he is formed, including the blood, possess this property." \*

De la Rive and Brunner,† later, suspending a bound-up frog between the poles of an electro-magnet, observed it to assume an equatorial position, thus realising Faraday's prediction that a complex animal organism must be diamagnetic in accordance with the properties of its constituent tissues and of the water which enters so largely into their composition.

Shortly after the publication of Faraday's researches on diamagnetism, Professor Plücker,‡ of Bonn, in a well-known paper, which appeared in 1848, after describing the characteristic behaviour of magnetic and diamagnetic liquids contained in watch-glasses placed upon and between the poles of powerful electro-magnets, gave the results of his observations on the diamagnetic properties of the blood. He not only confirmed, by experimenting on the blood of the frog, of man, and of the ox, the accuracy of Faraday's statements, but, by employing the microscope in his observations, he was able to show that the blood corpuscles are more strongly diamagnetic than the liquid in which they float.§

## 2. *Objects of the Present Investigation.*

At a time when all facts bearing on the physical properties and the chemical relations and structure of the blood-colouring matter are rightly claiming the attention of many of the leading workers in physiological chemistry, it appeared to me very desirable to examine the magnetic properties of the crystalline blood-colouring matter itself, in the condition of utmost available purity, and, whatever the results might be, to extend the inquiry to its leading iron-containing derivatives.

\* Faraday's 'Experimental Researches in Electricity,' vol. 3, p. 36 (2281).

† De la Rive and Brunner's researches are only known to me at second hand from the account given of them in Valentin's 'Grundriss der Physiologie' (4 Auflage, 1855, p. 507), where an engraving is reproduced in which a bound-up frog is shown placed between the poles of an electro-magnet.

‡ Plücker, 'Experimentelle Untersuchungen über die Wirkung der Magnete auf gasförmige und tropfbare Flüssigkeiten.' Refer to the heading "Über das magnetische und diamagnetische Verhalten der tropfbarflüssigen Körper," in 'Poggendorff's Annalen der Physik und Chemie,' vol. 73, 1848, p. 575, para. 49.

§ In 1874, Dr. R. C. Shettle, in a paper read before the Royal Society ('Roy. Soc. Proc.,' vol. 23, 1875, pp. 116–120), gave the results of experiments which had led him to the conclusion that arterial blood is paramagnetic as compared with venous blood, which is diamagnetic, the assumed difference in magnetic behaviour being explained by the author as due to the paramagnetic properties of the oxygen absorbed by venous blood. In reference to these statements, the only observation which I have to make, on the basis of my own work, is that they are entirely erroneous.

Although Faraday had shown that the blood is diamagnetic, and Plücker that the blood corpuscles are more decidedly diamagnetic than the liquid in which they float, it was yet conceivable, though improbable, that the iron-containing hæmoglobin would prove to be a feebly magnetic body, of which the true magnetic behaviour was concealed by the substances with which it is associated in the blood corpuscles. Whether hæmoglobin proved to be magnetic or diamagnetic, it obviously would be of great interest to examine the magnetic properties of the iron-containing substances which the blood-colouring matter yields when it is decomposed by acids in the presence of oxygen, and in the event of a difference between the magnetic behaviour of the mother-substance and its derivatives, to push the inquiry in a direction likely to lead to the discovery of the cause of the discrepancy. In pursuance of such an object I have been led to inquire whether the pure blood-colouring matter in aqueous solution is an electrolyte, and having discovered that it is one, to examine the results of its electrolysis. On this part of my inquiry the statements which I have to make in this paper are strictly preliminary, and, except in the first most interesting particular that oxy-hæmoglobin and CO-hæmoglobin separate in the first instance unchanged from their aqueous solutions when these are subjected to very feeble currents, are to be considered as liable to correction by future more extended work.

### *3. The Electro-magnet employed in the present Research.*

The electro-magnet employed was constructed by Ladd many years ago, and is sufficiently powerful to be employed for observations on the rotation of the plane of polarisation of light. I had it fitted with an accurately closing glass case and with adequate arrangements for the proper suspension of the bodies under examination. I am not possessed of instruments which would enable me to determine directly the strength of the magnetic field employed in my several sets of experiments. The Rev. F. J. Jervis-Smith, F.R.S., Millard Lecturer in Experimental Mechanics in the University of Oxford, to whom I had the pleasure of showing my experiments, had the great kindness to make careful measurements of the coils, and has practically reconstructed an electro-magnet similar in dimensions to mine, with the same windings, and of which the iron core derived from a similar instrument, made by Ladd, was probably identical in properties to that in my electro-magnet. Using Professor Rowland's method for determining the field, he obtained the following results:—

Intensity of the earth's horizontal magnetic component at Oxford = 0.18.

Distance between faces of pole pieces, 3 cm.

Current in Amperes.	Magnetic Field in C.G.S. units.
2·4	516
3·3	700
4·2	870

Probably it is safe as an approximate estimate to assume that my field was about 1000 C.G.S. units with a current of 5 amperes.

All the fundamental experiments on the magnetic properties of oxy-hæmoglobin, CO-hæmoglobin, and methæmoglobin were made by suspending cakes of the dried crystalline bodies by means of one or a few fibres of silk between the poles, thus avoiding the disturbing influence of glass tubes, however feebly magnetic. In the case of hæmin the substance was similarly examined, in the first instance, by suspending as far as possible rectangular cakes formed by the aggregation of microscopic crystals. In the case of hæmatin, the substance, being in an amorphous pulverulent condition, was necessarily examined in glass tubes, but its intensely magnetic properties prevented in its case, as in that of hæmin, any difficulties arising from the very feebly magnetic properties of the glass tube containing it.

#### 4. *Oxy-hæmoglobin a strongly Diamagnetic Body.*

The oxy-hæmoglobin employed in the present research was prepared by myself during the past winter from the blood of the horse by employing substantially the method of Hoppe-Seyler. In some cases the blood corpuscles were separated from the defibrinated blood by long continued centrifugalising; generally, however, the defibrinated blood was mixed with ten times its volume of a mixture made by diluting 10 volumes of saturated NaCl solution with 90 volumes of distilled water, and the corpuscles were then separated by means of the centrifuge.

In either case, the magma of corpuscles was treated with a small quantity of distilled water and pure ether, and the mixture having been thoroughly agitated in a stoppered separating funnel, the aqueous solution of the blood-colouring matter was separated and filtered into flasks surrounded with ice, and subsequently treated with one-fourth of its volume of pure absolute alcohol at a temperature of  $-5^{\circ}\text{C.}$ , and the mixture placed in an ice chamber for twenty-four or thirty-six hours. The oxy-hæmoglobin which crystallised was separated from its mother-liquor by means of the centrifuge. The crystalline mass was repeatedly washed with distilled water at  $0^{\circ}\text{C.}$ , and the washed crystals treated with distilled water at  $30^{\circ}\text{C.}$ ; the saturated solution

was rapidly centrifugalised, rapidly filtered into flasks surrounded by ice and salt, and the hæmoglobin caused to crystallise by the addition of absolute alcohol under the prolonged influence of cold. After being crystallised three times, the oxy-hæmoglobin was collected on filters, and the moist mass of microscopic crystals drained. The pasty crystalline mass was dried *in vacuo* over sulphuric acid at a temperature which never exceeded 5° C.

*Behaviour in the Magnetic Field.*—An irregular mass of three times crystallised oxy-hæmoglobin dried *in vacuo*, weighing 1·088 grammes, and measuring 18 mm. in length, 13 mm. in depth, and 13 mm. in breadth, was suspended by a couple of fibres of unspun silk between the poles of the magnet, the distance between these being 20 mm.; the mass was made to rest in the axial position before the current was passed through the coils.

Three cells of an accumulator were employed; on closing the key the mass of hæmoglobin instantly assumed the equatorial position. The experiment was repeated with masses of hæmoglobin prepared at various times, and recrystallised from one to three times, and weighing from 0·5 to 2 grammes, and invariably they were found to be powerfully diamagnetic.

A specimen of oxy-hæmoglobin of the horse, kindly prepared for me under the direction of Professor Hofmeister in the Chemico-Physiological Laboratory of the University of Strasburg, by the ammonium-sulphate method, and which had been five times crystallised, proved to be as powerfully diamagnetic as the oxy-hæmoglobin prepared by myself by Hoppe-Seyler's method.

5. *Carbonic-oxide-hæmoglobin is, like Oxy-hæmoglobin, strongly Diamagnetic.*

*Mode of Preparation.*—The carbonic oxide-hæmoglobin employed was prepared by saturating a concentrated solution of twice crystallised oxy-hæmoglobin with pure CO, and then crystallising the CO compound by the addition of absolute alcohol and exposure to a temperature of - 5°—10° C.

*Behaviour in the Magnetic Field.*—A nearly rectangular prismatic mass of CO-hæmoglobin which had been dried *in vacuo*, and which weighed 0·642 gramme, and of which the length was 17 mm., the breadth 6·5 mm., and the depth 13 mm., was brought into the axial position between the poles of the electro-magnet, the distance between these being 18 mm. On passing the current from three cells of an accumulator through the coils of the electro-magnet the mass instantly assumed the equatorial position. The experiment was repeated with different specimens of CO-hæmoglobin, and invariably with the same result.

In the absence of all data as to the diamagnetic moment of either Oxy- or CO-hæmoglobin, it is impossible to state whether these bodies differ in any degree in respect to their behaviour in the magnetic field. Working carefully but merely qualitatively, it would appear, however, that their behaviour in the magnetic field is identical.

6. *Methæmoglobin is, like Oxy-hæmoglobin, strongly Diamagnetic.*

The substance was prepared by adding to a saturated solution of twice crystallised oxy-hæmoglobin of the horse a few drops of solution of ferrieyanide of potassium until the characteristic change in colour and in the spectrum indicated the complete conversion into methæmoglobin. The solution was cooled to  $-5^{\circ}\text{C}$ ., treated with one-fourth of its volume of absolute alcohol at  $-10^{\circ}\text{C}$ ., and the mixture placed in ice and salt for a period of thirty-six hours. The crystalline methæmoglobin which separated was then washed with repeated quantities of ice-cold water, collected on a filter, drained, and dried *in vacuo* at a temperature not exceeding  $5^{\circ}\text{C}$ . Experiments with lumps of this substance varying in weight between 0.3 and 1.0 gramme showed it to be apparently as diamagnetic as oxy-hæmoglobin.

7. *Hæmatin and Acethæmin (Hæmin) intensely Magnetic Substances.*

*Preliminary Remarks.*

The more recent analysis of Jaquet, Zinoffsky, and Hüfner have led to the conclusion that, at any rate in the horse, the dog, the ox, and the hen, there exists a remarkable constancy in the proportion of the iron which exists in hæmoglobin (0.335 per cent.).\* If it be assumed that 1 molecule of hæmoglobin contains 1 atom of iron, the molecular weight of the hæmoglobin of the dog, the horse, the ox, and the hen would be 16,669, a result which concords admirably with the volume of oxygen and carbonic oxide which can enter into combination with hæmoglobin on the assumption (first of all advanced by Lothar Meyer) that 1 molecule of hæmoglobin can combine either with 1 molecule of oxygen or of carbonic oxide. The empirical formula for the hæmoglobin of the dog calculated by Jaquet from his analyses is probably very near the truth, namely,  $\text{C}_{758}\text{H}_{1203}\text{N}_{195}\text{S}_3\text{FeO}_{218}$ .

Why should hæmoglobin possess so enormously high a molecular weight? The question suggested itself to Bunge, who has furnished us with a reason which is eminently suggestive: "The enormous size of the hæmoglobin molecule," says this writer, "finds a teleological explanation, if we consider that iron is eight times as heavy as water.

\* For a discussion of all the more recent analysis of hæmoglobin, see my article on "Hæmoglobin" in Schäfer's 'Text-book of Physiology,' p. 199, *et seq*.

A compound of iron, which would float easily along with the blood current through the vessels, could only be secured by the iron being taken up by so large an organic molecule."

When oxy-hæmoglobin is subjected to the action of acids and alkalis it splits up with great ease into a coloured iron-containing body and into an albuminous body (or mixture of such bodies). The former, to which the name of Hæmatin has been given, is a derivative of the molecular group existing in the blood-colouring matter, upon which its colour, its spectroscopic characters, and its physiological properties doubtless depend, *though it is a derivative which is unquestionably a product of oxidation, and in no sense represents the real hæmochromogen*. According to Hoppe-Seyler, the empirical formula of hæmatin is  $C_{34}H_{35}O_5N_4Fe$ , whilst according to Nencki its composition is represented by the formula  $C_{32}H_{32}O_4N_4Fe$ .

When the decomposition of oxy-hæmoglobin is effected by glacial acetic acid in the presence of alkaline chlorides, a perfectly crystalline substance separates, which has been hitherto known under the name of *hæmin*, but which we shall now, following the suggestion of Nencki, term *acethæmin*. This body was looked upon by Hoppe-Seyler as a hydrochloride of hæmatin; the recent researches of Nencki and Zaleski have shown that acethæmin contains 8.59 of iron, and possesses a composition represented by the formula  $C_{34}H_{33}O_4N_4ClFe$ ; it contains an acetyl group, and both the acetyl and chlorine in it are linked to the iron. When this body is dissolved in weak solutions of sodium hydrate in the cold, the chlorine and acetyl are separated, and on neutralisation with acids, hæmatin of composition  $C_{32}H_{32}O_4N_4Fe$  is obtained. It is with these two coloured iron-containing decomposition products of hæmoglobin, hæmatin and acethæmin, that my observations have been carried out. Before referring to these in detail, I wish again to insist that these oxidation-products in no sense represent the unaltered iron-containing group to which the blood-colouring matter owes its physiological properties. As Hoppe-Seyler showed, when hæmoglobin is decomposed by acids and alkalis *in the absence of all traces of oxygen*, hæmatin is never formed, but a colouring matter which possesses the same spectrum as that which had previously been described by Stokes as that of reduced hæmatin.

This substance Hoppe-Seyler called hæmochromogen, and he expressed the opinion that it constitutes the veritable coloured radical upon which the physiological properties of hæmoglobin depend. The experimental facts advanced by Hoppe-Seyler have always appeared to me absolutely inadequate to warrant this hypothesis, which, however, is most suggestive, and demands a thorough and a new investigation.

*A. Magnetic Properties of Acethæmin.*

The acethæmin employed in the present research was prepared by me from ox's blood by the method of Schalfijew. Some of the specimens were purified by recrystallisation from glacial acetic acid, others by dissolving in a chloroformic solution of pure quinine, and subsequently adding to the filtrate hot glacial acetic acid, saturated with NaCl.\*

My first observations were made with a block of agglomerated hæmin crystals weighing 0.6455 gramme, and measuring 26 mm. in greatest length, 18 mm. in height, and 6 mm. in thickness: this block was suspended by two fibres of silk, so as to occupy the equatorial position in reference to the pole pieces of the magnet. The distance between the poles being 30 mm., on passing a current from three accumulator cells through the coils, the mass instantly assumed the axial position, and was strongly attracted to the nearest pole, the suspending silk fibres being sensibly deflected from their original vertical position. Even when the poles of the electro-magnet were 40 mm. apart, the mass instantly set in an axial position when the current was passed. The observations were repeated with numerous specimens of hæmin, and always with similar results.

*B. Magnetic Properties of Hæmatin.*

The hæmatin employed in these researches was prepared by dissolving recrystallised and perfectly pure acethæmin in a weak solution of chemically pure sodium hydrate at ordinary temperatures, and precipitating the filtered solution without delay by neutralising with dilute sulphuric acid. The precipitated hæmatin was thoroughly washed, drained, and dried. In consequence of its absolutely amorphous pulverulent character, my magnetic observations on this body were conducted with the aid of tubes of very feebly magnetic glass, containing from 0.1 to 0.4 of pure hæmatin. The intensely magnetic character of hæmatin was as easily demonstrated as had been that of acethæmin.

*8. Preliminary Observations on the Electrolysis of Solutions of Pure Oxy-hæmoglobin and CO-hæmoglobin.*

The remarkably definite results of my research, which had shown that Oxy- and CO-hæmoglobin are decidedly diamagnetic substances, whilst their iron-containing derivatives, acethæmin and hæmatin, are

\* Refer to my previously quoted article in Schäfer's 'Text-book of Physiology,' and to Nencki and Zaleski's recent article "Untersuchungen über den Blutfarbstoff. 1. Ueber die Aether des Hämins," 'Zeitschrift für Physiologische Chemie,' vol. 30, 1900, p. 384, *et seq.*



powerfully magnetic, naturally led me to speculate on the possible cause of these differences. It appeared to me that if hæmoglobin were found to be an electrolyte, apart from the interest which would attach to the discovery of the fact, a study of the products of its electrolysis might throw great light upon the question. Do we not know, for instance, that those compounds in which iron and other magnetic metals are present in electro-negative radicals are diamagnetic ?\*

In spite of my having made great efforts to purify as completely as possible the substances with which I worked, it is questionable whether their purity was sufficient for electrolytic researches. The experiments which I have yet made on this division of my subject must therefore be looked upon as strictly preliminary, and I hope in the course of the coming winter to extend them greatly, making use of compounds of hæmoglobin which have been subjected to far more frequent recrystallisation. In the course of these experiments, beside studying the proximate products of electrolysis with currents of different strength and potential, I intend to determine by the methods of Kohlrausch and Ostwald, with as great accuracy as possible, the specific conductivities of solutions of Oxy- and CO-hæmoglobin.

The following are the results of my electrolytic experiments which I wish at present to place on record :—

Firstly. When solutions of pure oxy-hæmoglobin are subjected to electrolysis at a temperature of about 15° C. between platinum electrodes, from twelve to sixteen cells of a carbon zinc bichromate battery being employed, and the current passing through the liquid being from 3 to 5 milliamperes, a rapid subsidence of the colouring matter takes place, the upper layers of the solution becoming perfectly colourless. The depositing colouring matter retains the spectroscopic character of oxy-hæmoglobin, and when stirred with it is absolutely and almost instantaneously soluble in the liquid from which it has separated. Exactly the same result occurs in the case of carbonic-oxide-hæmoglobin.

Secondly. On continuing the passage of the current through the solution in which precipitation has occurred, secondary reactions occur, gas is developed both at the anode and cathode, and in many cases a dirty white-brown deposit forms at the cathode.

Thirdly. Under conditions of strength of current and potential which were not determined with sufficient accuracy, and which I have not yet been able to reproduce at will, the solutions of oxy-hæmoglobin and CO-hæmoglobin have, under the long continued action of the current, on several occasions deposited at the anode an insoluble

\* W. Allen Miller, 'The Elements of Chemistry': Part I, "Chemical Physics," p. 422, London, 1855; H. du Bois, 'Propriétés magnétiques de la matière pondérable. Rapports présentés au Congrès International de Physique réunis à Paris en 1900,' Paris, 1901, Tome II, p. 460.

red colouring matter containing both the albuminous and the iron-containing residues of hæmoglobin. In the case of CO-hæmoglobin the compound deposited has presented the peculiar colour of CO-hæmoglobin.

*General Conclusions.*

The following are the conclusions to which I have been led by my experiments :—

1. The blood-colouring matter, oxy-hæmoglobin, as well as carbonic-oxide hæmoglobin and methæmoglobin, are decidedly diamagnetic bodies.

2. The iron-containing derivatives hæmatin and acethæmin are powerfully magnetic bodies. The differences in magnetic behaviour between the blood-colouring matter and acethæmin and hæmatin point to the profound transformation which occurs in the hæmoglobin molecule when it is decomposed in the presence of oxygen.

3. The preliminary study of the electrolysis of oxy-hæmoglobin and CO-hæmoglobin renders it probable that, in the blood-colouring matter, the iron-containing group, on which its physiological properties depend, is (or is contained in) an electro-negative radical: according to analogy, the iron in such a compound would possess diamagnetic and not magnetic properties.

In conclusion, I beg to acknowledge my indebtedness to Professor von Bunge, of Basel, to Professor Franz Hofmeister, of Strassburg, and to Dr. v. Ehrenberg, the technical director of the chemical factory of Messrs. Merck, of Darmstadt, for their great courtesy and kindness in placing at my disposal preparations of hæmoglobin prepared by themselves or under their direction. I have further to add that I reserve to myself the right of continuing without delay the researches of which the first results are contained in this paper.

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“On the Resistance and Electromotive Forces of the Electric Arc.” By W. DUDDELL, Whitworth Scholar. Communicated by Professor W. E. AYRTON, F.R.S. Received and Read June 20, 1901.

(Abstract.)

The discrimination between resistances and electromotive forces in conductors, or apparatus, in which both of these quantities are functions of the current is considered, and it is pointed out that whether such an apparatus may be said to possess a resistance, or an E.M.F., or both, depends to a large extent on the nature of the definition of these quantities, and a definition of these quantities is adopted.